Review

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Liver disease associated with canalicular transport defects: Current and future therapies

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Bile formation at the canalicular membrane is a delicate process. This is illustrated by inherited liver diseases due to mutations in *ATP8B1*, *ABCB11*, *ABCB4*, *ABCC2* and *ABCG5/8*, all encoding hepatocanalicular transporters. Effective treatment of these canalicular transport defects is a clinical and scientific challenge that is still ongoing. Current evidence indicates that ursodeoxycholic acid (UDCA) can be effective in selected patients with PFIC3 (ABCB4 deficiency), while rifampicin reduces pruritus in patients with PFIC1 (ATP8B1 deficiency) and PFIC2 (ABCB11 deficiency), and might abort cholestatic episodes in BRIC (mild ATP8B1 or ABCB11 deficiency). Cholestyramine is essential in the treatment of sitosterolemia (ABCG5/8 deficiency). Most patients with PFIC1 and PFIC2 will benefit from partial biliary drainage. Nevertheless liver transplantation is needed in a substantial proportion of these patients, as it is in PFIC3 patients. New developments in the treatment of canalicular transport defects by using nuclear receptors as a target, enhancing the expression of the mutated transporter protein by employing chaperones, or by mutation specific therapy show substantial promise. This review will focus on the therapy that is currently available as well as on those developments that are likely to influence clinical practice in the near future.

Keywords: PFIC; Therapy; Canalicular transport; Hereditary cholestasis.

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Abbreviations: ABC, adenosine triphosphate-binding-cassette; PC, phosphatidylcholine; PS, phosphatidylserine; PFIC, progressive familial intrahepatic cholestasis; BRIC, benign recurrent intrahepatic cholestasis; GGT, gamma-glutamyl transpeptidase; ICP, intrahepatic cholestasis of pregnancy; LPAC, low-phospholipid associated cholelithiasis syndrome; DJS, Dubin Johnson syndrome; UDCA, ursodeoxycholic acid; PXR, pregnane X receptor; MARS, extracorporal albumin dialysis; PBD, partial biliary diversion; IB, ileal bypass; PEBD, partial external biliary diversion; PIBD, partial internal biliary diversion; NBD, nasobiliary drainage; FXR, farnesoid X receptor; 6-ECDCA, 6-ethyl chenodeoxycholic acid; PPAR, peroxisome proliferator activator receptor; CAR, constitutive androstane receptor; CF, cystic fibrosis; 4-PBA, 4-phenylbutyrate acid; CFTR, cystic fibrosis transmembrane conductance regulator.

Introduction

The process of primary bile formation occurs at the canalicular membrane predominantly through the action of transporters belonging to the adenosine triphosphate-binding-cassette (ABC) family [1,2] (Fig. 1A). Secretion of bile salts, phosphatidylcholine (PC) and cholesterol is mediated by ABCB11 (BSEP), ABCB4 (MDR3) and ABCG5/8, respectively. Excretion of organic anions is mediated by other members of the ABC-family such as ABCC2 (MRP2). In addition, ATP8B1 (FIC1), a P4 P-type ATPase, is essential for a proper composition of the canalicular membrane, and thus for normal bile flow [3].

Bile formation at the canalicular membrane is a delicate process and any inaccuracy may have devastating consequences. This is illustrated by inherited liver diseases caused by mutations in any of the hepatocanalicular transporters described above. In this review, we give recommendations for the treatment of canalicular transport defects based on current evidence. In addition, this review focuses on the developments that are likely to influence clinical practice in the near future.

Canalicular transport defects in liver disease

Phospholipid-flippases ATP8B1 (FIC1)

ATP8B1 is thought to specifically translocate phosphatidylserine (PS) from the outer to the inner leaflet of plasma membranes, causing the outer leaflet to be enriched in PC, sphingomyelin and cholesterol [4–7]. Cholesterol has a high affinity for sphingomyelin, and both are thought to be preferentially located in laterally separated microdomains (previously called lipid rafts). These microdomains offer protection against the detergent action of bile salts in the canalicular lumen and are essential for normal function of transmembrane transporters [8–10]. Recent evidence indicates that canalicular ABC-transporters are indeed localized within these microdomains [11]; therefore, disruption of lipid asymmetry and reduction of cholesterol content in the apical membrane decreases the function of resident proteins such as the bile salt export pump ABCB11, resulting in cholestasis [9,10]. In addition, the canalicular membrane in both humans and mice with ATP8B1/Atp8b1 deficiency develops a decreased

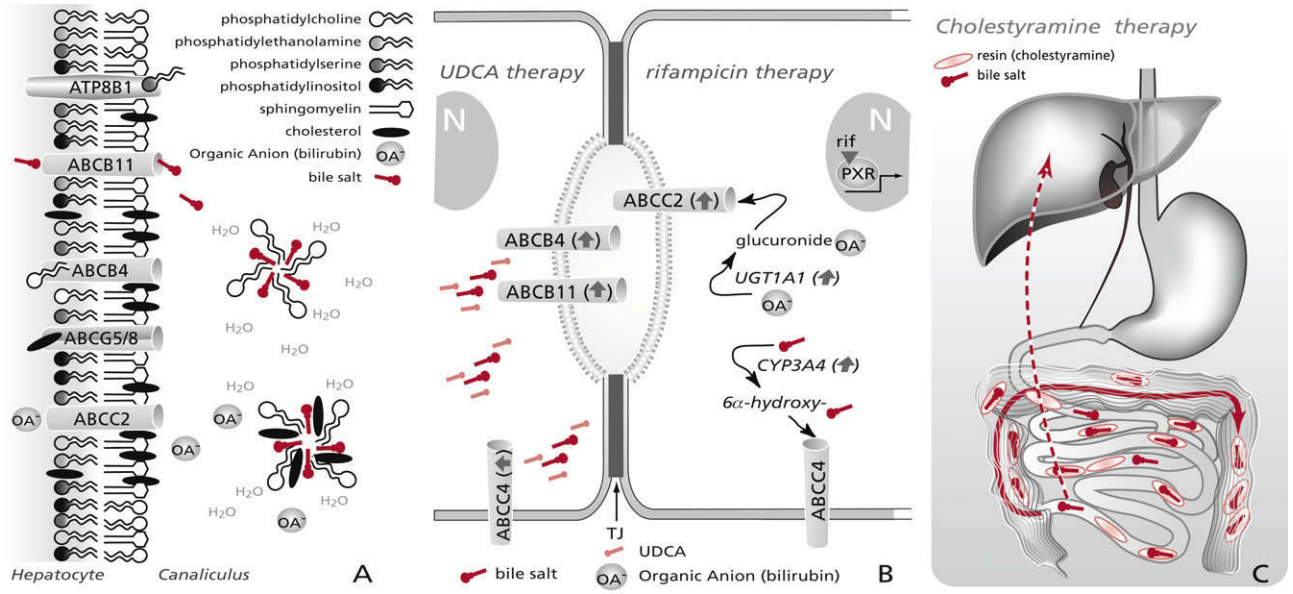

Fig. 1. Medical treatment of canalicular transport defects. (A) Schematic representation of bile formation at the canalicular membrane. ATP8B1 (FIC1) is essential for normal bile flow, probably through maintaining an asymmetric distribution of phospholipids between the inner and outer leaflet of the canalicular membrane. Secretion of bile salts into the canaliculus by the bile salt export pump ABCB11 (BSEP) is the main driving force for bile flow, with water following through osmotic forces. ABCB4 (MDR3) and ABCG5/8 induce secretion of phosphatidylcholine and cholesterol, respectively. These lipids form mixed micelles with the bile salts and protect membranes lining the biliary tract against detergent bile salts. ABCC2 (MRP2) mediates efflux of a broad range of organic anions. As indicated most ABC transporters are probably organized in microdomains enriched in sphingomyelin and cholesterol. (B) Left panel: the effect of UDCA in the hepatocyte. The hydrophilic ursodeoxycholic acid (UDCA) partly replaces the endogenous cytotoxic hydrophobic bile salts. In addition, by inducing expression of ABCB11, and ABCB4, UDCA stimulates hepatobiliary secretion of bile salts and protective phospholipids. The up-regulation of ABCC4 (MRP4) induces the efflux of conjugated bile acids across the basolateral membrane. Right panel: rifampicin (RIF) activates PXR regulated transcription of CYP3A4. This stimulates 6α-hydroxylation of bile salts, which can be excreted at the basolateral membrane via ABCC4 (MRP4), with subsequent excretion in the urine. In addition, the conjugation and excretion of bilirubin is enhanced through induction of UGT1A1 and ABCC2 (MRP2). (C) Cholestyramine binds bile salts in the intestinal lumen and interrupts the enterohepatic circulation of bile salts by reducing re-absorption and stimulating faecal excretion. N, nucleus; TJ, tight junction.

resistance to hydrophobic bile salts, evidenced by an enhanced biliary recovery of phospholipids, cholesterol and canalicular ectoenzymes in bile [9,12,13]. It is likely that this damage to the canalicular membrane adds to the cholestasis.

ATP8B1 deficiency (formerly FIC1 disease) is an autosomal recessive condition characterized by mutations in the *ATP8B1* gene [3,14,15]. Patients with ATP8B1 deficiency may present in infancy or early childhood with progressive familial intrahepatic cholestasis type 1 (PFIC1) [15–18] or later in life with episodes of cholestasis and intractable pruritus: benign recurrent intrahepatic cholestasis type 1 (BRIC1) [19–22]. PFIC1 and BRIC1 are in fact two ends of a clinical spectrum, as is illustrated by patients who initially present with episodic cholestasis but progress to permanent cholestasis in time [14,23,24]. During a cholestatic episode, all patients have low serum concentrations of gamma-glutamyl transpeptidase (GGT) in combination with high serum bile salt levels. Liver biopsies of PFIC1 patients show bland cholestasis with characteristic coarse and granular bile on the ultrastructural level [12,25]. ATP8B1 is localized on the canalicular membrane of the hepatocyte [6,26,27], but its expression is even more abundant in other tissues [3,28], where it can also be found at the apical membranes of polarized cells [26,27,29]. This is consistent with a proposed general cellular function of ATP8B1 and with extrahepatic features such as persistent short stature [16], diarrhoea [16,30,31], pancreatitis [22,32], sensorineural hearing loss [29,33] and an abnormal sweat composition [16,32], which are frequently present in patients with ATP8B1 deficiency. Heterozygous mutations in *ATP8B1* can be found in patients with intrahepatic cholestasis of pregnancy (ICP), a liver disorder that is characterized by pruritus and raised serum bile salt levels during pregnancy or use of oral contraceptives [34,35] (Table 1).

Bile salt transporter ABCB11 (BSEP)

Since bile flow is largely dependent on bile salt excretion, it is not surprising that a deficiency of ABCB11, the main bile salt transporter, can cause a severe autosomal recessive cholestatic syndrome that is hard to distinguish from ATP8B1 deficiency. Patients may present with progressive intrahepatic cholestasis in the first decade of life that rapidly leads to liver failure (PFIC2) [36,37]. However, ABCB11 deficiency also represents a phenotypic spectrum, with episodic cholestasis (BRIC2) as the mild manifestation [38–41]. Biochemically, serum concentrations of bile salts are markedly elevated, but GGT concentrations remain low [25,42]. Histological characteristics of the liver, with portal-tract fibrosis, bile duct proliferation and amorphous canalicular bile may distinguish ABCB11 from ATP8B deficiency [25]. In addition, ABCB11/Abcb11 localization is restricted to the canalicular membrane of hepatocytes [36,43,44] and no extrahepatic symptoms are described. In contrast, cholelithiasis is often observed, probably due to biliary bile salt concentrations that are too low to solubilise all biliary cholesterol [38,41]. Also, hepatocellular carcinoma or cholangiocarcinoma may be a complication of ABCB11 deficiency [45,46]. Heterozygous mutations in *ABCB11*

Table 1. Canalicular transporters and canalicular transport defects.

Canalicular transporter (synonym)	Canalicular transport defect (synonym)	Disease characteristics	Biochemical and histological characteristics	Disease associated with heterozygous canalicular transport defect
ATP8B1 (FIC1)	**ATP8B1 deficiency** (FIC1 disease, PFIC1, Byler disease and Greenland familial cholestasis, BRIC1, Tygstrup-Summerskill and Walshe cholestasis)	Spectrum of intrahepatic cholestasis comprising PFIC1 and BRIC1 **PFIC1**: progressive intrahepatic cholestasis, pruritus and in some patients extrahepatic symptoms **BRIC1**: episodic cholestasis, pruritus and in some patients extrahepatic symptoms. In between episodes no symptoms	High serum bile salts but low GGT concentrations. Liver biopsy: bland cholestasis with coarse and granular bile	ICP
ABCB11 (BSEP)	**ABCB11 deficiency** (PFIC2, BRIC2)	Spectrum of intrahepatic cholestasis comprising PFIC2 and BRIC2 **PFIC2**: progressive intrahepatic cholestasis, pruritus and in some patients cholelithiasis **BRIC2**: episodic cholestasis, pruritus and in some patients cholelithiasis. In between episodes no symptoms	High serum bile salts but low GGT concentrations. Liver biopsy: portal-tract fibrosis, bile duct proliferation and amorphous canalicular bile	ICP, drug induced cholestasis, transient neonatal cholestasis
ABCB4 (MDR3)	**ABCB4 deficiency** (PFIC3)	Progressive intrahepatic cholestasis, high serum GGT concentrations. Pruritus less prominent	High serum bile salts and high GGT concentrations. Liver biopsy: fibrosis and marked bile duct proliferation	ICP, drug induced cholestasis, transient neonatal cholestasis LPAC
ABCC2 (MRP2)	**Dubin Johnson syndrome**	Asymptomatic but in some patients gastrointestinal symptoms	High serum conjugated bilirubin concentrations. Liver biopsy: dark blue or black due to pigmentation	
ABCG5/8	**Sitosterolemia**	Xanthomas, arthralgias and premature coronary artery disease	High serum sitosterols with relatively low cholesterol concentration. Liver biopsy: unknown	

PFIC, progressive familial intrahepatic cholestasis; BRIC, benign recurrent intrahepatic cholestasis; GGT, gamma-glutamyl transpeptidase; ICP, intrahepatic cholestasis of pregnancy; LPAC, low-phospholipid associated cholelithiasis syndrome.

are described in drug induced cholestasis [47], ICP [48–50] and transient neonatal cholestasis [51] (Table 1).

Phosphatidylcholine transporter ABCB4 (MDR3)

ABCB4 is expressed at the apical membrane of the hepatocyte [44,52] and is essential for PC secretion into the bile [53,54]. A defective ABCB4 protein causes an imbalance in the composition of primary bile, with lack of PC and a surplus of bile salts, the latter damaging the canaliculus and small bile ducts, causing chronic and progressive liver disease [55]. Mutations in *ABCB4* are associated with progressive familial intrahepatic cholestasis type 3 (PFIC3) which, like the other PFIC types, inherits in an autosomal recessive pattern [55,56]. In contrast to patients with PFIC1 and PFIC2, serum GGT levels are elevated. Liver histology reveals fibrosis (progressing to cirrhosis) and marked bile duct proliferation [55]. There are no extrahepatic symptoms but heterozygous mutations can be encountered in unexplained cholestasis [57,58] and many milder cholestatic conditions, such as ICP [59,50,60–66], drug induced cholestasis [47], transient neonatal cholestasis [67], and isolated and recurrent intrahepatic cholesterol gallstones, designated as LPAC (low-phospholipid associated cholelithiasis syndrome) [68–71] (Table 1). The latter is characterized by gallstone disease at relatively young age (<40 yrs) persistent after cholecystectomy. The underlying mechanism is an insufficient concentration of PC in bile to form mixed micelles with cholesterol, resulting in cholesterol supersaturation and crystal formation. The wide clinical spectrum of ABCB4 deficiency is illustrated by a patient with a heterozygous mutation in *ABCB4* described by Lucena et al. This patient presented with juvenile cholelithiasis, recurrently manifested ICP and finally developed biliary cirrhosis [72].

Organic anion transporter ABCC2 (MRP2)

ABCC2 expression is found in the liver but also at the apical membranes of other polarized cells [44,73,74]. Its substrate specificity is broad and comprises organic anions, mainly conjugated compounds. ABCC2 has an important role in the excretion of bilirubin into bile and in the excretion of bile salts after their sulfation or glucuronidation [75,76]. Nevertheless, the mild hepatic phenotype and lack of extrahepatic symptoms in ABCC2 deficiency suggests that other transporters can complement its function. ABCC2 deficiency causes Dubin Johnson syndrome (DJS). This syndrome is an autosomal, recessively inherited disorder characterized by chronic or intermittent conjugated hyperbilirubinemia. Although some patients complain about gastrointestinal symptoms and drug metabolism might be different, there are no further symptoms. Plasma concentrations of liver enzymes are usually within the normal range, and there is no permanent liver damage. However, on macroscopic examination the liver itself appears dark blue or black due to pigmentation [77–79] (Table 1). So far, no associations between the heterozygous state and liver or other disease has been found [80].

Cholesterol transporter ABCG5/8

ABCG5/8 is expressed at the apical membrane of liver and intestine [81]. The protein-complex consists of two half transporters, ABCG5 and ABCG8, that heterodimerise in the endoplasmic reticulum to become functionally active [82,83]. The ABCG5/8 transporter has a major role in the biliary and intestinal excretion of cholesterol and plant sterols (mainly sitosterols) [84,85]. Mutations in either *ABCG5* or *ABCG8* cause a rare autosomal recessive disease, sitosterolaemia. This disease is characterized by an

increased retention of sitosterols by the intestine and a failure to secrete sterols into bile, resulting in high plasma sitosterol levels and accumulation of sterols in peripheral tissues and blood [84–87]. Patients consequently present with tendon xanthomas, arthralgias and premature coronary artery disease, despite relatively low plasma levels of cholesterol [88–90] (Table 1). Sporadically, haemolytic abnormalities are mentioned [91]. Except for one patient with chronic active hepatitis and signs of cirrhosis, nothing is known about liver histology [92]. The effect of being a heterozygous carrier for these mutations is not clear yet [93].

Treatment of canalicular transport defects

All liver diseases described above are due to mutations in genes encoding hepatocanalicular transporters. For most of these diseases the response to current medical therapy is either non-existent or of limited duration. Some agents have proven to be effective in specific situations, mainly by providing symptomatic relief.

Nevertheless, most patients with progressive cholestasis eventually need surgical intervention. Even patients with the relative "benign" phenotypes of intrahepatic cholestasis (BRIC) may undergo invasive therapy, purely to improve quality of life [16,22,24].

Current medical treatment

The therapeutic strategies for cholestasis due to canalicular transport defects may target bile composition, bile salt toxicity and the secretion of bile salts. The ideal therapy should have anti-cholestatic, anti-fibrotic and anti-neoplastic properties. Ursodeoxycholic acid (UDCA), rifampicin and cholestyramine are amongst the most commonly used. Sometimes combination therapy is employed, but there is no evidence for any synergistic effect.

UDCA

The main therapeutic target of UDCA is the protection of hepatocytes and cholangiocytes by replacing endogenous, cytotoxic bile salts [94,95]. In addition, UDCA induces expression of functional transporters at transcriptional and post-transcriptional level and enhances bile flow, possibly through cholehepatic shunting [96–101]. A simplified illustration of the effect of UDCA in the hepatocyte can be found in Fig. 1B.

More than half of the patients with high GGT-PFIC or proven PFIC3 responded to UDCA treatment [102–104] (Table 2A). Although in most reports this response was not further clarified we presume that it was characterized by at least partial improvement in serum transaminase levels and pruritus. Interestingly, those with missense mutations generally had a good response to UDCA therapy, while those with a premature stop codon showed no response [103]. Therefore in patients with PFIC3 and a presumed residual function of the ABCB4 protein based on mutational analysis, UDCA is the therapy of choice.

In patients with low GGT-PFIC or an undefined subtype of PFIC, the response to UDCA therapy was much less promising. Although in some reports serum transaminase levels and pruritus improved in about half of the patients upon UDCA [102,104,105], in most studies UDCA was not effective [106–113]. Even in patients with a mild phenotype (BRIC1, BRIC2), UDCA did not prevent or abort a cholestatic attack [19,24,31,39,40,114–116] (Table 2A). Currently it is not possible to predict who would benefit from UDCA in low GGT cholestasis and it is doubtful whether UDCA has a place in the treatment of ATP8B1 or ABCB11 deficiency. Especially for patients with progression to severe liver disease, surgical management is needed as soon as possible.

Table 2A. UDCA treatment in hepatocanalicular transport defects.

Hepatocanalicular defect	Number patients	Outcome (number patients)	Reference
PFIC undefined subtypes	58	Improvement (2) Partial improvement (0) No improvement (56)	[107,111]
Low GGT-PFIC	98	Improvement (22) Partial improvement (12) No improvement (64)	[102,104–106, 108–110,112,113]
High GGT-PFIC	46	Improvement (20) Partial improvement (14) No improvement (12)	[102–104]
BRIC	12	Improvement (0) Partial improvement (2) No improvement (10)	[19,24,31,39, 40,114–116]
DJS	2	Improvement (1) Partial improvement (0) No improvement (1)	[77,117]

"Improvement", indicates (almost complete) normalization of serum transaminases and/or bilirubin concentration and total relief of pruritus.
"Partial improvement", indicates no complete normalization of serum transaminases and/or bilirubin concentration with or without persistent pruritus.
"No improvement", indicates no response or deterioration of the symptoms.
PFIC, progressive familial intrahepatic cholestasis; BRIC, benign recurrent intrahepatic cholestasis; DJS, Dubin Johnson syndrome; GGT, gamma-glutamyl transpeptidase. In the high GGT-PFIC subtype, 11 patient are reported twice [102,103].

For DJS, two case-reports have been published with opposite effect. In one patient, serum bilirubin declined upon UDCA treatment, while in the other patient a combination of rifampicin with UDCA led to a dramatic rise in serum bilirubin and bile salt concentrations, which normalized once again after these medications were discontinued [77,117] (Table 2A). For sitosterolaemia no clinical trials or case-reports have been published.

UDCA treatment is safe, and except for the reversible effect in the DJS patient no serious side-effects have been described.

Recently it was found that shortening of the side chains increases the therapeutic efficacy of UDCA [100]. This modified, so called *nor*UDCA has already been proven to be more effective than the parent compound in a murine model of primary sclerosing cholangitis [118].

Rifampicin

The primary effect of rifampicin is inducing CYP3A4 expression through activation of the xenosensor pregnane X receptor (PXR). This increases 6α-hydroxylation of bile salts, compounds which can subsequently be glucuronidated and excreted in the urine [99,119]. In addition, conjugation and excretion of bilirubin is enhanced through induction of UGT1A1 and ABCC2 [99]. Enhanced expression of the latter might also be important for excretion of other, still unidentified pruritogenic compounds (Fig. 1B).

In patients with low GGT-PFIC, rifampicin did not have any long lasting effect on serum concentration of bilirubin and transaminases, but reduced the pruritus in some patients [108,109, 112,113]. This marginal effect contrasts with the results obtained in patients with BRIC. After starting rifampicin treatment in seven patients, eighteen out of twenty-two episodes were completely aborted within several weeks [24,115,116,120] (Table 2B). Thus

it seems that rifampicin can reduce pruritus in some patients with low GGT-PFIC, but might even induce remission in patients with BRIC. Nevertheless, rifampicin treatment should be used with caution because of its potential hepatotoxic effect.

The use of rifampicin in DJS has been described in one patient, but instead of reducing serum bilirubin concentrations, the conjugated bilirubinemia increased during treatment [77] (Table 2B). It is not known whether rifampicin affects cholesterol homeostasis as well, but so far no experience with sitosterolaemia has been published.

Cholestyramine

Cholestyramine is a negative ion exchange resin that binds bile salts in the intestinal lumen, reduces re-absorption and stimulates faecal excretion of bile salts (Fig. 1C) [121].

Cholestyramine does not seem to be effective in patients with low GGT-PFIC or undefined subtypes of PFIC [107,108,112,122,123]. For patients with BRIC the results are variable, varying from shortening of the cholestatic episodes [24,124,125] to no effect at all [19,24,115,116,126] (Table 2C). Consequently cholestyramine seems to have no place in the treatment of PFIC, but it may be beneficial in patients with BRIC. Given the potentially better tolerability and higher efficacy of the new bile salt resin binders with other polymer structures, such as colesevalam [127], these drugs should be the topic of further investigations.

There is no published evidence for cholestyramine treatment in high GGT cholestasis or DJS. However, extensive experiments are available for sitosterolaemia in which cholestyramine in combination with a diet low in cholesterol reduced the serum levels of plant sterols with improvement of clinical symptoms, such as reduction of xanthomas [88,93,128–132] (Table 2C). Chronic cholestyramine treatment may cause constipation, but no other serious complications have been found.

Invasive treatment

A few BRIC patients have been treated successfully with extracorporal albumin dialysis (MARS) [133,134]. However biliary diversion and liver transplantation are the most commonly used invasive treatments.

Table 2B. Rifampicin treatment in hepatocanalicular transport defects.

Hepatocanalicular defect	Number patients	Outcome (number patients)	Reference
Low GGT-PFIC	17	Improvement (0) Partial improvement (3) No improvement (14)	[108,109,112,113]
BRIC	7	Improvement (5) Partial improvement (0) No improvement (2)	[24,115,116,120]
DJS	1	Improvement (0) Partial improvement (0) No improvement (1)	[77]

"Improvement", indicates the almost complete normalization of serum transaminases and/or bilirubin concentration and total relief of pruritus. For patients with BRIC "improvement" indicates the abortion of a cholestatic attack. "Partial improvement" indicates lack of complete normalization of serum transaminase concentration but improvement of pruritus. "No improvement" indicates lack of response or deterioration of the symptoms.
PFIC, progressive familial intrahepatic cholestasis; BRIC, benign recurrent intrahepatic cholestasis; DJS, Dubin Johnson syndrome; GGT, gamma-glutamyl transpeptidase.

Table 2C. Cholestyramine treatment in hepatocanalicular transport defects.

Hepatocanalicular defect	Number patients	Outcome (number patients)	Reference
PFIC undefined subtypes	23	Improvement (0) Partial improvement (1) No improvement (22)	[107,122,123]
Low GGT-PFIC	34	Improvement (0) Partial improvement (0) No improvement (34)	[108,112]
BRIC	20	Improvement (2) Partial improvement (2) No improvement (16)	[19,24,115,116,124–126]
Sitosterolaemia	13	Improvement (12) Partial improvement (1) No improvement (0)	[88,93,128–132,210]

"Improvement" indicates an almost complete normalization of serum transaminases, bilirubin and/or sterol concentration and total relief of pruritus. In BRIC "improvement" indicates the abortion of a cholestatic attack. "Partial improvement" indicates a lack of complete normalization of serum transaminases, bilirubin and/or sterol concentration with or without persistent pruritus. In patients with BRIC, "partial improvement" means reduction of pruritus. "No improvement" indicates a lack of response or deterioration of the symptoms.
PFIC, progressive familial intrahepatic cholestasis; BRIC, benign recurrent intrahepatic cholestasis; GGT, gamma-glutamyl transpeptidase.

Biliary diversion

Non-transplant surgical intervention can be effective in patients with intrahepatic cholestasis. Partial biliary diversion (PBD) or ileal bypass (IB) are two of these surgical interventions, in which PBD may be achieved by either a jejunal conduit from gallbladder to the abdominal wall (partial external biliary diversion; PEBD) [123], or one that connects the gallbladder to the colon (partial internal biliary diversion; PIBD) [109]. Leaving the common bile duct intact, PBD establish only a partial diversion of bile (about 80%) while the remainder enters the duodenum. In IB, bile salt re-absorption is diminished by bypassing the small intestine at the terminal ileum through an ileocolonic anastomosis (Fig. 2) [135]. Although to a much stronger extent, the working mechanism of PBD is similar to cholestyramine – it reduces the accumulation of toxic bile salts by a reduction of the enterohepatic circulation.

PEBD was initially described by Withington and Withington in 1988 [123]. This innovative technique was quickly adopted by other centres worldwide and so far sixteen additional case-reports/series addressing the effect and technique of PEBD in the treatment of PFIC have been published (Table 3). Except for one small series of five patients in which PEBD did not have any effect [104], all others report normalization or improvement of liver function in 75–100% of the patients with low GGT-PFIC, indicated by at least improved liver tests and reduced pruritus [106,108,110,112,136–143]. The response in patients with an undefined subtype of PFIC seems to be less [111,144]. Liver biopsies post PEBD were performed in some patients and did not show further progression or even a resolution of hepatic morphologic abnormalities in all these patients [112,123,137,141]. Advanced disease and liver cirrhosis were proposed as the main causes of therapeutic failures, indicating that early surgical intervention in PFIC patients is essential. Three BRIC1 patients were treated with PEBD to improve quality of life rather than to prevent disease progression. In these patients PEBD aborted the cholestatic attack immediately but did not always prevent subsequent minor cholestatic episodes [24,116].

Another surgical technique for biliary drainage is IB, first described by Whitington et al. [108] in patients who were not

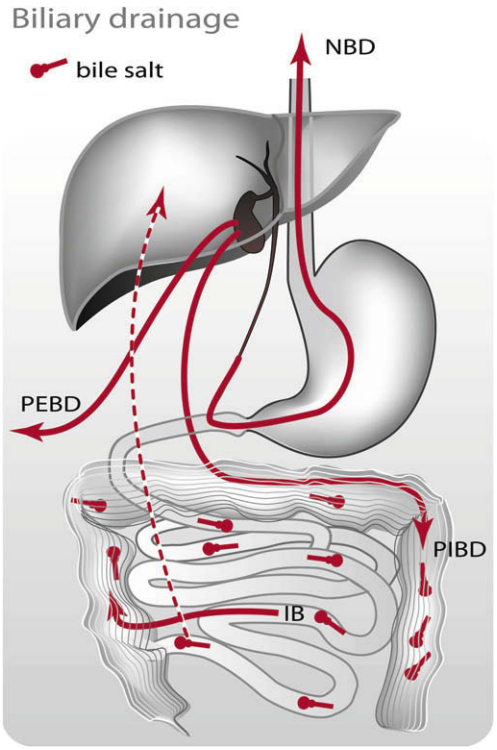

Fig. 2. Biliary drainage. Partial biliary diversion (PBD), or ileal bypass (IB), are two of non-transplant surgical interventions that interrupt the enterohepatic circulation of bile salts and can be effective in the treatment of canalicular transport defects. PBD may be achieved by either a jejunal conduit from gallbladder to the abdominal wall: partial external biliary diversion; PEBD, or one that connects the gallbladder to the colon: partial internal biliary diversion; PIBD. In IB, bile salt re-absorption is diminished by bypassing the terminal ileum through an ileocolonic anastomosis. A cholestatic attack in patients with BRIC may be aborted by endoscopically introducing a nasobiliary drain during a cholestatic episode (NBD).

Table 3. Partial biliary drainage in hepatocanalicular transport defects.

Hepatocanalicular defect	Number treated	Outcome (number patients)	Reference
PFIC undefined subtypes **Treatment by PEBD**	42	Improvement (24) Partial improvement (5) No improvement (13)	[111,123,144]
Low GGT-PFIC **Treatment by PEBD**	94	Improvement (66) Partial improvement (11) No improvement (17)	[104,106,108, 110,112,136–143]
High GGT-PFIC **Treatment by PEBD**	1	Improvement (0) Partial improvement (0) No improvement (1)	[104]
BRIC **Treatment by PEBD**	3	Improvement (1) Partial improvement (2) No improvement (0)	[24,116]
PFIC undefined subtypes **Treatment by IB**	5	Improvement (1) Partial improvement (0) No improvement (4)	[144]
Low GGT-PFIC **Treatment by IB**	7	Improvement (6) Partial improvement (0) No improvement (1)	[108,135]
Low GGT-PFIC **Treatment by PIBD**	2	Improvement (2) Partial improvement (0) No improvement (0)	[109]
BRIC **Treatment by PIBD**	1	Improvement (1) Partial improvement (0) No improvement (0)	[109]

"Improvement" indicates an almost complete normalization of serum transaminases and/or bilirubin concentration and total relief of pruritus. "Partial improvement" indicates a lack of complete normalization of serum transaminases and/or bilirubin concentration and/or persistent pruritus. "No improvement" indicates a lack of response or deterioration of the symptoms.
PFIC, progressive familial intrahepatic cholestasis; BRIC, benign recurrent intrahepatic cholestasis; GGT, gamma-glutamyl transpeptidase; PEBD, partial external biliary diversion; PIBD, partial internal biliary diversion; IB, ileal bypass.
In the low GGT-PFIC subtype, four patient are reported double [108,123].

amenable for PEBD because of a previous cholecystectomy. An additional advantage of this procedure is the lack of an external fistula. However, after a short initial response, clinical symptoms recurred in half of the treated patients with low GGT- or an undefined subtype of PFIC within a year [135,144] (Table 3). This is probably due to secondary adaptation of the ileum to the resection and it was therefore concluded that IB is inferior to PEBD in patients with low GGT cholestasis.

Recently, PIBD has been described in two teenage patients with PFIC and low GGT cholestasis. This technique combines the advantages of external drainage and ileal bypassing by partially interrupting enterohepatic circulation without an external biliary fistula. The initial clinical and laboratory results were very promising, but long-term follow-up is necessary to evaluate late results and complications [109] (Table 3).

In the few BRIC patients treated with PEBD, drainage immediately reduced pruritus and relieved cholestasis. Moreover, in the follow-up period just a few very short additional episodes were noticed [24,116] (Table 3). However the permanent character of PEBD makes it less appropriate to be used in a disorder that is only episodic. Based on these results we developed a temporary intervention: nasobiliary drainage (NBD) for which the first results were published in 2006 [145]. Until now, in our centre a total of twelve cholestatic attacks in five BRIC1 patients were treated by NBD. This was established by endoscopically introducing a nasobiliary drain during a cholestatic episode (Fig. 2). In eight out of twelve treatments, pruritus totally resolved within 48 h and serum bile salt levels returned to normal or near normal levels. Failure of NBD was either due to practical difficulties when introducing the drain (one treatment) or progression of liver disease (three treatments in two patients who are now doing well after PEBD). Thus, for most cholestatic episodes in BRIC1, NBD is an effective therapy. Because in some patients there is a transition from episodic to progressive cholestasis, PEBD should be considered when NBD fails to resolve a cholestatic episode.

Given the current evidence, PBD is the therapy of choice in patients with low GGT-PFIC, and should be performed as soon as possible after diagnosis to prevent liver damage. At present, large multicenter studies are in progress that investigate PBD results stratified for patients with ATP8B1 vs ABCB11 deficiency and in subpopulations with different mutations. It is to be expected that the current recommendations can be refined upon publication of these results. It is also important to realize that PBD induces the loss of considerable amounts of fluids and electrolytes, and patients might become dehydrated. Adequate and individualized electrolyte supplementations and fluid is mandatory in all patients with PBD. Finally complications from intestinal surgery as stoma prolaps and intestinal obstruction have been described.

There is no evidence of the effect of non-transplant surgery in patients with other canalicular transport defects, except for one

patient with ABCB4 deficiency who was unsuccessfully treated with PEBD [104].

Liver transplantation

Because of the high risk of complications and the life-long need for immune-suppressive therapy, liver transplantation should be reserved for patients who have established liver cirrhosis at the time of presentation or who have progressive liver disease despite treatment. Unfortunately, for many patients with the severe form of ATP8B1, ABCB11 and ABCB4 deficiency, liver transplantation is still the only option.

Independent of the subtype of PFIC, the survival rate after transplantation ranges from 61–92% in the 80ths and early 90ths [108,137,146] to 75–100% more recently [31,104,106,111,147–154]. However, most follow-up periods do not yet exceed 3 years. Transplantation improved cholestasis-related symptoms like itching, malnutrition and liver function in almost all surviving patients. Due to the lack of cadaver donors, living-related liver transplantation is often used in patients with PFIC. Although it was feared that the heterozygous status of the parent donor would affect the results unfavourably, complications and survival rates of these types of transplantation in PFIC patients are similar to living-related transplantations for non-genetic liver diseases such as biliary atresia [149,151]. However, in some PFIC patients, symptoms of cholestasis may recur after several years. Apart from the usual long-term complications after liver transplantation, such as chronic rejection and conduit stricture, in PFIC this may also be due to allo-immunization of the recipient against the ATP8B1, ABCB11 or ABCB4 protein located in the (heterozygous) donor liver [155].

The ATP8B1 protein is abundantly expressed outside the liver, e.g. in the intestine [3,27] and correction of the liver defect by transplantation will not cure the extrahepatic symptoms, such as diarrhoea. Indeed, in a substantial proportion of PFIC1 patients, diarrhoea exacerbates when biliary bile salt secretion is restored after liver transplantation. In these patients, liver biopsies revealed severe steatosis [31,147,148,150,154]. In one case, by constructing a total biliary diversion after transplantation, all bile salts were diverted from the intestine and diarrhoea resolved [153].

The ABCG5/8 half transporters are also expressed in both intestine and liver but, in contrast to ATP8B1 deficiency, liver transplantation was completely effective in normalization of sterol plasma concentration in a patient with sitosterolaemia, suggesting that adequate excretion of sterols by the liver is sufficient for keeping levels of xenosterols low, even when absorption in the intestine remains increased [92].

Recently, transplantation of human hepatocytes has been used for treatment of liver-based metabolic conditions. The injected hepatocytes would in theory have a selective growth advantage over the patient's own defective hepatocytes and should (partly) repopulate the native liver, as has already been shown in a mouse model for PFIC [156]. Unfortunately, for the two PFIC2 patients treated so far there was no clear benefit, probably due to pre-existing fibrosis that precluded proper engraftment of hepatocytes into the liver [157,158].

Future treatment options

As previously described, mutations in the canalicular transporters cause defective bile formation and retention of substances which are normally secreted into the bile. This may be due to a decreased transporter expression at the plasma membrane, due to a functional defect of the protein itself or a combination of both possibilities. It follows that ameliorating the expression of functional protein at the canalicular membrane could theoretically restore bile flow and improve liver disease in some patients.

Nuclear receptors as therapeutic target

Bile formation is highly regulated by nuclear receptors such as the bile salt sensor farnesoid X receptor (FXR). FXR is activated by naturally occurring (chenodeoxycholic acid) or synthetic (GW4064 and 6-ethyl chenodeoxycholic acid (6-ECDCA)) ligands. Upon activation it transactivates a number of genes that co-ordinately reduce hepatic bile salt uptake and *neo*synthesis and stimulate bile salt detoxification and secretion of both bile salts and the protective phospholipids [159–162] (Fig. 3). Synthetic ligands for FXR and other nuclear receptors are increasingly recognized as possible therapeutic options in cholestatic syndromes [163]. The use of FXR as a target for drug-therapy has already been studied in some detail in several rat models for cholestasis. For example, treatment with potent synthetic FXR ligands protected rats against oestrogen-induced cholestasis [164]. The latter condition has been linked to reduced activity and/or diminished expression of several transporters at the canalicular membrane, including Abcb11 and Abcc2 [165,166]. This new class of drugs is currently investigated in patients with primary biliary cirrhosis [163]. We propose that this treatment would also positively affect the cholestasis associated with the canalicular transport defects reviewed in this article.

ATP8B1 is not a known target gene for any of the nuclear receptors but its activity was described to influence FXR function. Although controversial [7,167,168], ATP8B1 deficiency might directly or indirectly reduce FXR expression and activity in liver and intestine [169–173]. The resulting down-regulation of *ABCB11* would be an explanation for the cholestatic phenotype in ATP8B1 deficient patients. Hence, if synthetic FXR ligands could counteract the FXR down-regulation this would induce *BSEP* expression and improve canalicular transport of bile salts. However, until now this concept has only been tested in cell culture [173].

In addition to FXR ligands, ligands of other nuclear receptors have been proven to affect the expression of canalicular transporters as well. As the classic ligands for peroxisome proliferator activator receptor alpha (PPARα), fibrates directly increase the expression of Abcb4/ABCB4 at the canalicular membrane and induce PC secretion [174,175]. For fibrates, clinical trials in cholestatic diseases such as primary sclerosing cholangitis, primary biliary cirrhosis and chronic hepatitis C have been started, and initial results are promising [176–179]. The nuclear constitutive androstane receptor (Car) and Pxr share the same response element in the rat *Abcc2* promotor with Fxr. Ligands for Car and PXR, such as phenobarbital and rifampicin, induce *Abcc2/ABCC2* expression [99,161]. These nuclear receptor ligands are already used to treat liver disease due to canalicular transport defects with variable results as discussed above for rifampicin.

Mutation specific therapy

Inserting a non-mutated gene or correcting a mutation at the DNA level was long considered to be the Holy Grail in the treatment of genetic diseases. Unfortunately, safe and effective gene therapy turned out to be much harder to accomplish than expected. Some of the early trials in humans resulted in mortality and enthusiasm for this approach decreased [180]. However, recent advances in our understanding of transcription, transla-

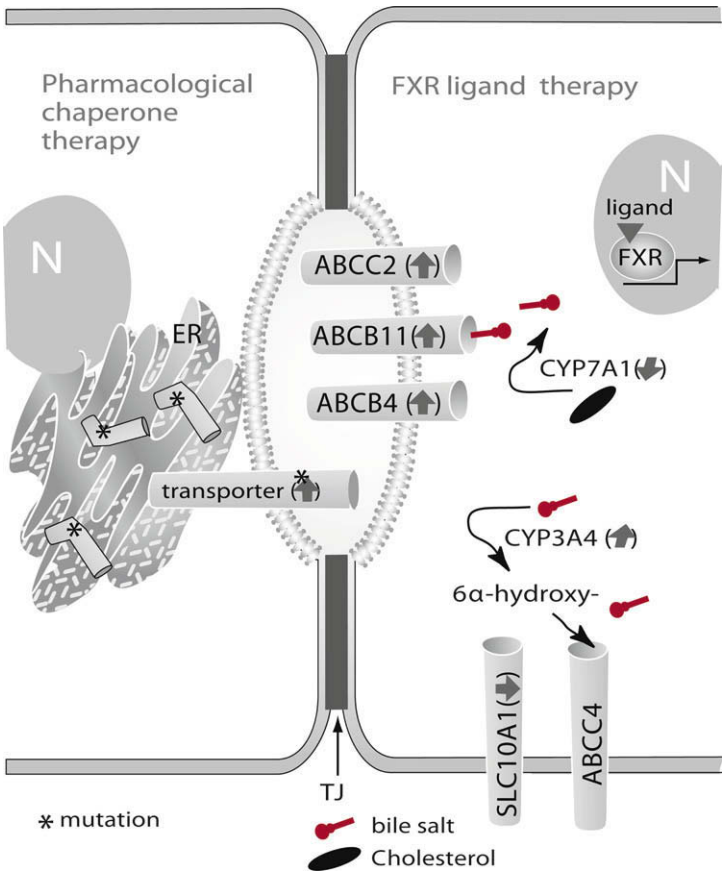

Fig. 3. Future treatment of canalicular transport defects. Left panel: many missense mutations influence protein processing, causing the abnormal but potentially functional protein to be misfolded, trapped in the ER and subsequently degraded. Pharmacological chaperones (such as 4-phenylbutyrate acid (4-PBA)) are small molecular weight compounds that help stabilize these abnormal proteins and enhance the expression of transporters at the canalicular membrane. Right panel: artificial ligands (such as GW4064) activate FXR. FXR transactivates a number of genes that together coordinate bile salt homeostasis. Reduced transcription of the sodium/bile acid co-transporter SLC10A1 (NTCP) decreases the bile salt uptake at the basolateral membrane. *Neo*synthesis of bile salts is reduced by inhibition of the transcription of the rate limiting enzymes (for example CYP7A1) in the conversion of cholesterol to bile salts. CYP3A4 is induced, enhancing 6α-hydroxylation of bile salts and subsequent excretion through ABCC4. Finally, FXR activation causes increased secretion of bile salts (through induced ABCB11 (BSEP) expression), phospholipids (through induced ABCB4 (MDR3) expression) and bilirubin (through induced ABCC2 (MRP2) expression). N, nucleus; ER, endoplasmic reticulum; TJ, tight junction.

tion and the subsequent processing of proteins have opened the possibility to obtain functional protein at the right place, even when mutations are still present in the DNA. This exciting new development has recently moved from bench to bedside in some early phase I and II trials, mainly in cystic fibrosis (CF). This approach should be applicable to other genetic diseases, including canalicular transport defects.

It has been known for many years that aminoglycosides, in addition to their antimicrobial activity, can suppress premature termination codons. [181,182]. Results from clinical trials with gentamicin in CF patients with premature stop codons due to nonsense mutations were promising [183,184]. However, the potential toxicities and its insufficient oral absorption precluded wide spread use of this antibiotic. As it was already clear that premature stop codons could be suppressed, a large number of compounds were screened to find such a drug. The first of this new group of drugs to become available was PTC124 [185]. After supportive results in a phase-I study in healthy volunteers [186], a phase-II study was recently started in CF patients, which shows normalization of chloride transport in about half of all patients [187]. We expect that PTC124 can also ameliorate the phenotype in patients with hepatocanalicular transport defects. This treatment would however be restricted to a subpopulation of patients in whom the disease is caused by specific premature stopcodons (e.g. UGA).

When missense mutations are present at an ATP binding site, or in a functional domain, the resulting protein is generally dysfunctional. However, many missense mutations influence protein processing, causing the abnormal but potentially functional protein to be misfolded, trapped in the endoplasmic reticulum and subsequently degraded. Pharmacological chaperones are small molecular weight compounds that help stabilize these abnormal proteins (Fig. 3) [188]. 4-Phenylbutyrate acid (4-PBA) is such a pharmacological chaperone which is already approved by the US Food and Drug Administration for use in urea-cycle disorders, where it acts as an ammonia scavenger [189,190]. The working mechanism probably involves interfering of 4-PBA with degradation and maturation of the mutated proteins, by modulation of the heat shock protein expression [191–193] (Fig. 3). The application of 4-PBA to protein-misfolding diseases was first studied for the deltaF508 mutation in the cystic fibrosis transmembrane conductance regulator (*CFTR)* gene. *In vitro* treatment of nasal- and bronchial epithelial cell lines resulted in an increased expression of mature CFTR at the plasma membrane and restoration of chloride secretion [194]. Clinical trials

Table 4. Recommendations for treatment of hepatocanalicular transport defects.

Hepatocanalicular defect	Recommendation for treatment
PFIC1–2 (low GGT-cholestasis)	PEBD should be performed directly after diagnosis. Consider liver transplantation when this treatment fails
PFIC3 (high GGT-cholestasis)	Start UDCA treatment as soon as possible. Consider liver transplantation when this treatment fails
BRIC 1–2	Start with rifampicin early at the beginning of an episode (cave hepatotoxicity). For some patients the addition of cholestyramine may be favourable. When no improvement in serum bile salts levels and pruritus is seen within 4–8 weeks perform NBD. If both medical therapy and NBD are not effective, consider PEBD
DJS	No specific therapy. Drug metabolism might be different in DJS
Sitosterolemia	Low-sterol-diet in combination with cholestyramine

PFIC, progressive familial intrahepatic cholestasis; BRIC, benign recurrent intrahepatic cholestasis; DJS, Dubin Johnson syndrome; GGT, gamma-glutamyl transpeptidase; PEBD, partial biliary drainage; UDCA, ursodeoxycholic acid; NBD, nasobiliary drainage.

with 4-PBA (Buphenyl) in patients with a homozygous deltaF508 mutation in the *CFTR* gene show improvement of chloride transport in the nasal epithelia [195,196]. Several mutations in *ATP8B1* [197], *ABCB11* [198] and *ABCB4* [199] have been shown to influence proper protein folding *in vitro*. These findings indicate that strategies to stabilize the mutant protein at the canalicular membrane by 4-PBA or other pharmacological chaperones may be therapeutic in patients with hepatocanalicular transport defects as well. 4-PBA has been tested *in vitro* for the E297G and D482G mutations frequently found in ABCB11 deficiency. Treatment reduced the protein ubiquitination and increased the cell surface expression of mature ABCB11 [200–202]. Plasma membrane expression of mutated ATP8B1 could also be induced by 4-PBA [197].

Mutation specific therapy of splice-site mutations can be divided into two groups. Some splice-site mutations generate both aberrantly and correctly spliced transcripts; if the latter are present the resulting disease is generally not severe [203,204]. The variability of splicing patterns is regulated through the interaction of a complex repertoire of splicing factors [205] which implies molecular targets for mutation specific therapy. Indeed, compounds like sodium butyrate can enhance the expression of the full-length transcripts in the presence of splice-site mutations [206]. Next, *in vivo* studies should confirm the benefit of splicing factor inducers in monogenetic diseases. The other group of splicing mutations completely abolish exon recognition. Although some early *in vitro* work shows that this category of mutations might also be amenable to mutation specific treatment, many issues have still to be solved [207–209].

Conclusion

The hepatocanalicular transport defects underscore the essential role of these transporters in bile formation. At present, surgery (PBD or liver transplantation) is the only effective therapy in most patients. Although medical treatment seems to be effective in some patients this is not true for others. Based on current evidence [211] and our own experience we propose different treatment options for the specific canalicular transport defects as depicted in Table 4. It should be clear that a universally effective and non-invasive treatment for patients afflicted by canalicular transport defects still has to be developed. However, with the scientific progress in the field of nuclear receptor ligands and mutation specific therapy, this might be accomplished within the next decade.

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